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Induced and spontaneous colitis mouse models reveal complex interactions between IL-10 and IL-12/IL-23 pathways

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ABSTRACT

Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD). These idiopathic and chronic diseases result from inflammation of the gastrointestinal tract and are mainly mediated by the immune system. Genome wide association studies link genes of the IL-12 and IL-23 biology to both CD and UC susceptibility. IL-12 and IL-23 cytokines share a functional subunit, p40, and their respective receptors also share a functional subunit, IL-12Rβ1. However, clinical trials targeting p40, and thus inhibiting both IL-12 and IL-23 pathways, provided mitigated effects on IBD, suggesting context dependent effects for each cytokine. In addition to IL-12 and IL-23, genetic deficiencies in IL-10 also result in severe IBD pathology. We generated various mouse models to determine how IL-12 or IL-23 interacts with IL-10 in IBD pathology. Whereas defects in both IL-10 and IL-12R do not impact the severity of the Dextran Sulfate Sodium (DSS)-induced colitis, combined deficiencies in both IL-10 and IL-23R aggravate the disease. In contrast to DSS-induced colitis, defects in IL-12R and IL-23R both protect from the spontaneous colitis observed in $IL10^{-/-}$ mice. Together, these studies exemplify the complexity of genetic and environmental interactions for identifying biological pathways predictive of pathological inflammatory processes.

1. Introduction

In the past decade, inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), have emerged as a worldwide public health challenge, with over 1.5 million individuals suffering from IBD in North America and Europe alone [1]. IBD is a complex disease with both genetic and environmental factors contributing to its development, and it is characterized by inflammation of the intestinal tract. Overproduction of inflammatory cytokines plays a pivotal role in IBD pathogenesis, where IL-12, IL-23 and IL-10 are key players [2].

Both IBD protective and deleterious polymorphism in the IL-12 and IL-23 signaling pathways have been identified in humans, highlighting the importance of these two cytokines in IBD [3–7]. The IL-12 and the IL-23 cytokines share the p40 subunit while their respective receptor share the IL-12Rβ1 chain [8], suggesting that these cytokines may share common signaling pathways. However, while activation of the IL-12/IL-12 receptor signaling pathway drives the Th1 response and

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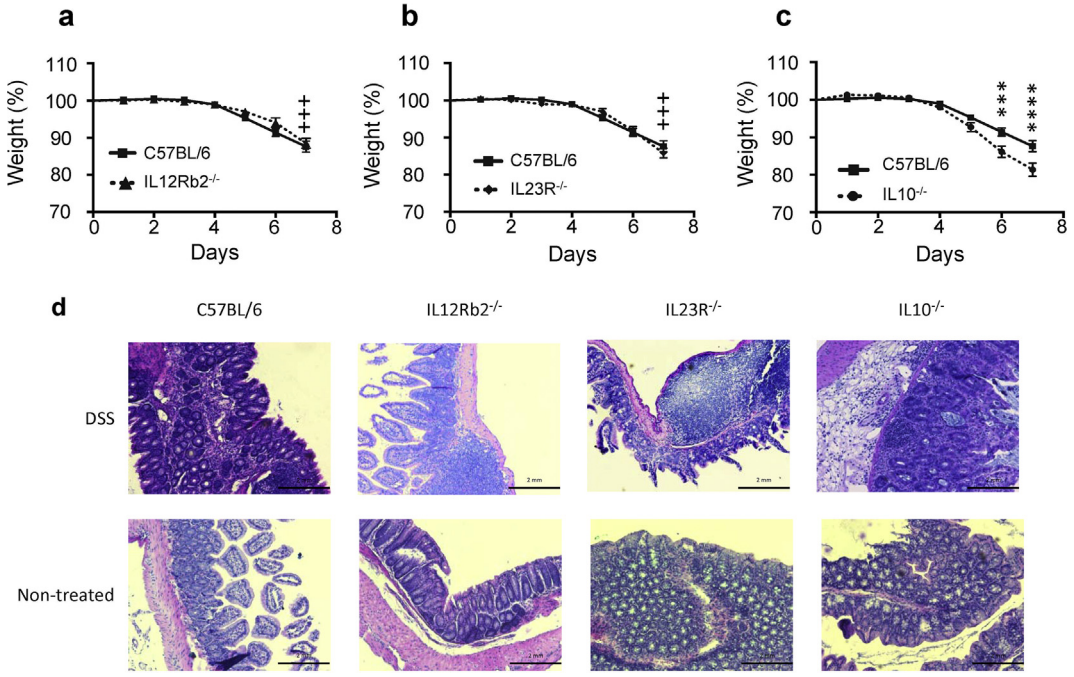

Fig. 1. IL-10 deficiency exacerbates DSS-mediated colitis. Mice were treated with 3% DSS for 7 days. Body weight was determined daily in control C57BL/6 (black square, n > 17 mice) versus (a) IL12Rb2$^{-/-}$ (triangle, n = 16 mice), (b) IL23R$^{-/-}$ (diamond, n = 15) and (c) IL10$^{-/-}$ mice (circle, n = 13). Data are represented as mean ± SEM. For panels a and b, "+++" denotes a *P* value < 0.001 for significant weight loss relative to day 0. For panel c, *P* values of *** < 0.005, **** < 0.001 for significant weight loss of IL10$^{-/-}$ mice relative to C57BL/6 mice at the indicated time points. (d) Representative images of H&E-stained colon sections comparing the degree of inflammatory and degenerative lesions in C57BL/6, IL12Rb2$^{-/-}$, IL23R$^{-/-}$ and IL10$^{-/-}$ mice after 7 days of DSS administration. Depending on the mouse strain, DSS-induced different grades of mucosal damage, immune cell infiltration, and goblet cell loss (100×).

production of IFN-γ, the IL-23/IL-23 receptor signaling pathway drives the Th17 response leading to the production of IL-17A, IL-17F and IL-22 cytokines [8]. In addition, we have recently shown that the IL-12 and IL-23 receptors are expressed on different immune cells and their activation is associated with distinct inflammatory responses [9]. Yet, both IL-12 and IL-23 can exacerbate intestinal inflammation. For instance, in the Dextran Sulfate Sodium (DSS) colitis mouse model, injection of IL-12 cDNA increased inflammation and lead to acceleration and aggravation of colitis [10]. Conversely, administration of an anti-p40 vaccine, blocking both IL-12 and IL-23, reduced chronic intestinal inflammation and improved clinical score of DSS-treated mice [11]. Antibodies against IL-23p19 also suppress chronic intestinal inflammation in *Helicobacter hepaticus*-infected RAG knockout mice [12]. Thus, both IL-12 and IL-23 cytokines seem to play significant, yet distinct, roles in IBD pathogenesis.

In addition to IL-12 and IL-23, IL-10 represents another key signaling pathway involved in intestinal homeostasis and IBD pathogenesis [13]. Polymorphisms in the IL-10/IL-10R pathway are associated with higher risk of developing CD and UC in humans, and specific variants in *IL10R* cause a more severe form of colitis [14,15]. IL-10 is secreted by a wide variety of immune cells including macrophages, dendritic cells and T cells and plays a protective role through suppression of the Th1/Th17 pathogenic response important for intestinal homeostasis [16–19]. In the absence of IL-10, there is an increase in naive T differentiation into Th1 or Th17 and production of inflammatory cytokines causing spontaneous colitis [20].

There is some evidence that both IL-12 and IL-23 can modulate colitis development in IL10$^{-/-}$ mice. For instance, administration of anti-IL-12 antibody to IL10$^{-/-}$ mice decreases intestinal inflammation and protects from disease development [21]. In addition, reduction of IL-12 and IL-23 levels following treatment with irsogladine maleate prevented colitis in IL10$^{-/-}$ mice [22], whereas administration of IL-23 accelerates colitis development in these mice [23]. However, despite evidence of these interactions between IL-10 and the IL-12 and IL-23 signaling pathways, our knowledge about their combined effect on IBD development as well as the nature of the inflammatory response underlying these effects is limited. Thus, our goal was to examine the relationship between IL-10 and the IL-12/IL-23 signaling pathways, in otherwise immune-competent mice, to understand their specific effect and their cumulative impact on the development of intestinal inflammation and IBD. Specifically, we investigated the relationship between these pathways under two inflammatory models: chemically-induced colitis, which disrupts the epithelial barrier, and spontaneous inflammatory colitis, driven by the absence of immunoregulation.

2. Methods

2.1. Mice

All mice were maintained on C57BL/6 (B6; #664) background. B6 and IL-10-deficient (IL10$^{-/-}$; #2251) mice as well as mice deficient for the beta 2 chain of the IL-12 receptor (IL12Rb2$^{-/-}$; #3248) were purchased from The Jackson Laboratory. IL-23 receptor-deficient mice (IL23R$^{-/-}$) mice bear an IRES-GFP cassette after exon 8 of the endogenous gene leading to its deletion in homozygotes animals [24], and were generously provided from Dr. Kuchroo based at Harvard Medical School and Brigham and Women's Hospital. We generated double knock-out (DKO) mice by crossing IL10$^{-/-}$ mice with either IL12Rb2$^{-/-}$ or IL23R$^{-/-}$ mice yielding both IL10$^{-/-}$.IL12Rb2$^{-/-}$ and IL10$^{-/-}$.IL23R$^{-/-}$ DKO mice. All of these strains were maintained at the Maisonneuve-Rosemont Hospital animal housing facility (Montreal, Canada). The Maisonneuve-Rosemont Hospital ethics committee, overseen by the Canadian Council for Animal Protection, approved the experimental procedures.

2.2. DSS-induced and IL10$^{-/-}$-spontaneous colitis models

Colitis was induced in male mice six to twelve weeks of age by

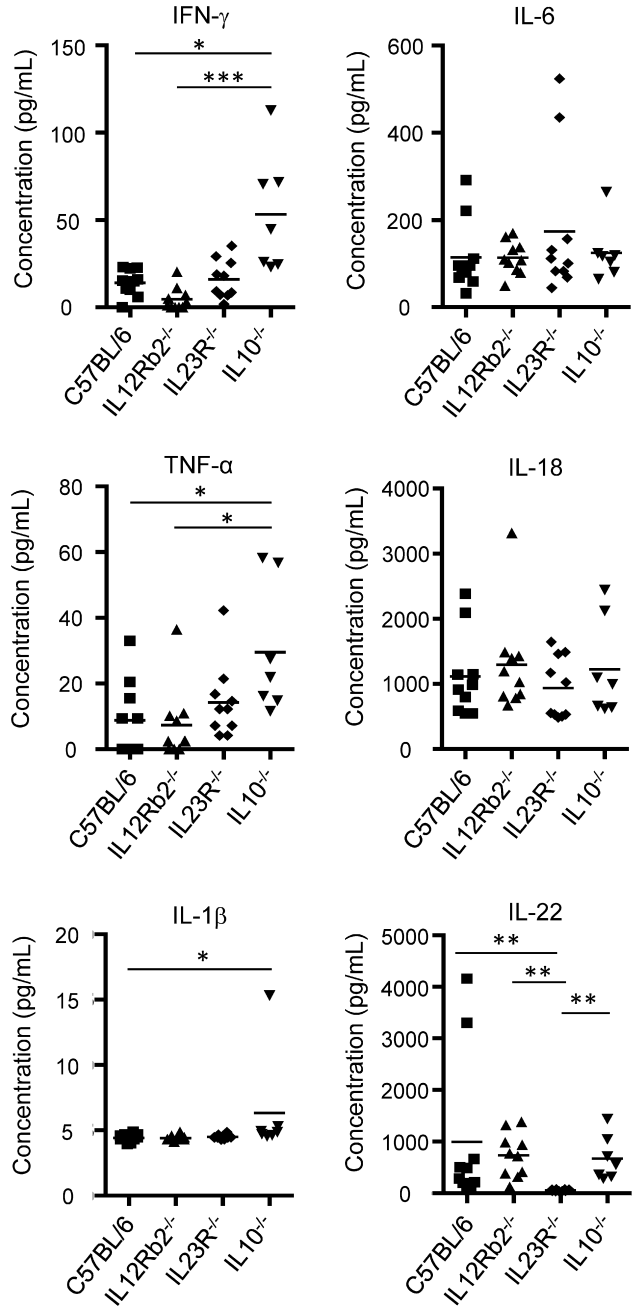

Fig. 2. IL10$^{-/-}$ mice display increased levels of pro-inflammatory cytokines. Cytokine levels of C57BL/6 (square, n = 10), IL12Rb2$^{-/-}$ (triangle, n = 10), IL23R$^{-/-}$ (diamond, n = 10) and IL10$^{-/-}$ (inverted triangle, n = 7) were measured in serum of DSS-treated mice using the Cytokine 17-Plex Mouse ProcartaPlex™. For all panels, the horizontal bar represents the mean for each group and the dot represents one mouse sample. *P* values, $^{*} < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$. For IL-22, when the two elevated data points for C57BL/6 mice are removed from the analysis, the difference in IL-22 expression between C57BL/6 and IL23R$^{-/-}$ mice is no longer significant, although the tendency remains (data not shown). IL-1β levels remain statistically elevated in IL10$^{-/-}$ mice relative to C57BL/6 mice, even upon removing the elevated data point for IL-1β in IL10$^{-/-}$ mice (data not shown).

adding 3% (wt/vol) DSS (#160110, MP Biomedicals) in the drinking water. Each mouse cohort was paired based on weight and age. The mice were euthanized after 7 days of DSS treatment. For spontaneous colitis, we followed a cohort of male mice for at least 25 weeks. These mice received non-acidified water, in order to maintain a normal intestinal microbial flora [25]. For DSS-induced colitis, mice were monitored daily for weight loss, hunched posture, dehydration, stools (diarrhea, blood) and rectal prolapse. IL10$^{-/-}$ mice were monitored daily for hunched posture and dehydration and every two weeks for weight loss, stools (diarrhea, blood) and rectal prolapse. With the approval of our ethics committee, mice were euthanized when they lost more than 25% of their body weight or when they presented a moribund appearance.

2.3. Quantification of serum cytokines

Mouse serum was collected at day 7 in the DSS-induced colitis model or in mice older than 25 weeks of age in the spontaneous colitis model. We used the Th1/Th2/Th9/Th17/Th22/Treg Cytokine 17-Plex Mouse ProcartaPlex™ Panel (EPX170-26087-901, ThermoFisher Scientific) to quantify the following cytokines: IFN-γ, GM-CSF, TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-22, IL-23, IL-27. Not all analytes were detectable. In mice subjected to DSS-induced colitis, we detected 6 analytes and in the spontaneous colitis model, we detected 9 cytokines. The threshold of detection is based on the standard curves and the assay sensitivity based on the manufacturer's instructions.

2.4. Histology

DSS-treated mice were euthanized at day 7 and colon sections were dissected and thoroughly rinsed before samples were fixed in 10% neutral-buffered formalin for 24 h and embedded in paraffin. Paraffin sections (5 µm) were prepared from each block and observed under a Leica DMIL LED microscope; representative photos were taken with a Leica DFC490 camera and acquired with LAS software v4.5. Histopathological evaluation of colon tissue was based on Mahler et al. [26].

2.5. Statistics

Significance was tested with a one-way ANOVA with Bonferroni post-hoc test or with the non-parametric Kruskal-Wallis followed by a Dunn's multiple comparison test, as appropriate. In some cases, analyses were performed with and without data outliers as described in the result section. A two-way ANOVA was used when testing for significant difference between 2 groups over time. Significance was accepted at $P < 0.05$.

3. Results

3.1. IL-12Rβ2 and IL-23R deficiency do not provide resistance to DSS-mediated IBD

IL-10, IL-12 and IL-23 represent key independent pathways involved in the etiology of IBD. To understand the contribution of each of these cytokines to the inflammatory response, we first compared the response to DSS-induced colitis in control B6 mice and mice deficient for IL-12Rβ2, IL-23R or IL-10. In parallel with previous reports [27,28], DSS induced a significant weight loss in B6, IL12Rb2$^{-/-}$ and IL23R$^{-/-}$ mice, reaching a ~10% weight loss at day 7 in these three groups of mice (Fig. 1a and b). In contrast, IL10$^{-/-}$ mice exhibited significantly more weight loss compared to B6 mice reaching almost 20% by day 7 (Fig. 1c). We next evaluated whether the weight loss was paralleled by damages to the colon. Histopathological evaluation showed no colon damage in all groups of control mice, whereas upon DSS-treatment, all four mouse strains showed some degree of mucosal damage, goblet cell loss as well as immune cell infiltration, where the degree of immune cell infiltration was more pronounced in IL10$^{-/-}$ mice (Fig. 1d). We also observed enhanced arborization of crypts and oedema of the submucosa (Fig. 1d and Supplementary Fig. 1). Together, these results suggest that deficiency in either IL-12Rβ2 or IL-23R signaling does not protect from

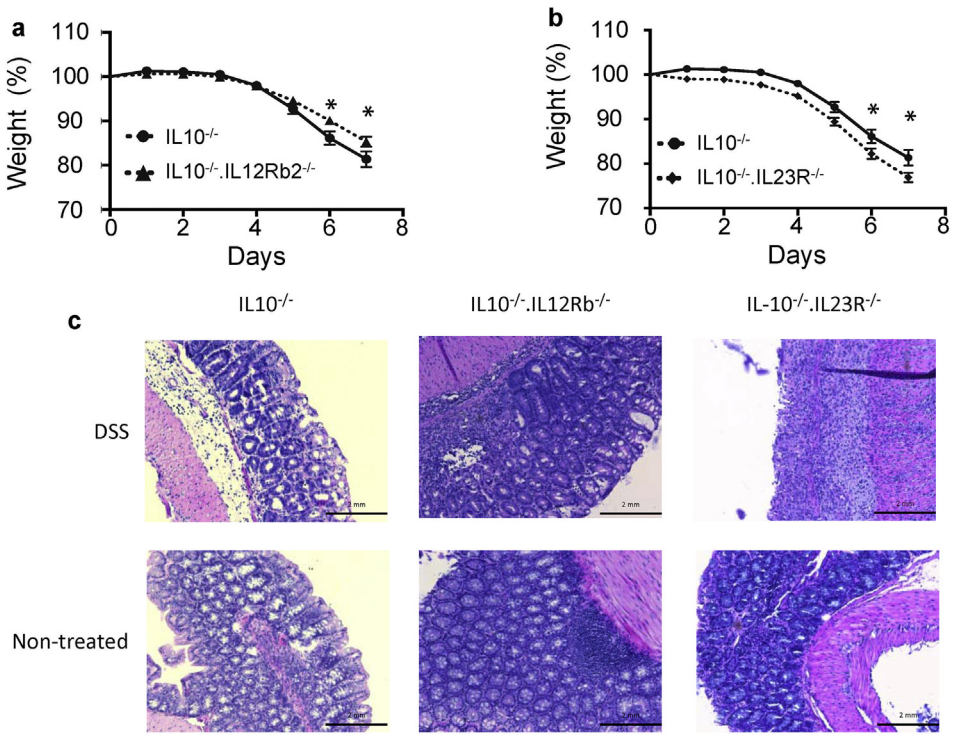

Fig. 3. IL-23R deficiency exacerbates DSS-mediated colitis in IL10$^{-/-}$ mice. Mice were treated with 3% DSS for 7 days. Body weight was determined daily in IL10$^{-/-}$ (circle, n = 13 mice) versus (a) IL10$^{-/-}$.IL12Rb2$^{-/-}$ (triangle, n = 10 mice) or (b) IL10$^{-/-}$.IL23R$^{-/-}$ (diamond, n = 10 mice). Data are represented as mean ± SEM. *P* values, $^{*} < 0.05$. (c) Histological analysis of acute DSS colitis in IL10$^{-/-}$, IL10$^{-/-}$.IL12Rb2$^{-/-}$ and IL10$^{-/-}$.IL23R$^{-/-}$ mice by H&E-stained colon sections. Different degrees of immune cell infiltration and goblet cell loss are observed in DSS-treated mice, with evident ulceration in IL10$^{-/-}$.IL23R$^{-/-}$ mice (100×).

DSS-induced colitis and that IL-10 deficiency exacerbates weight loss and colon damage. This is consistent with studies showing that IL10$^{-/-}$ mice are more susceptible to DSS induced colitis [29,30].

3.2. The systemic inflammatory response to DSS treatment is pathway-specific

To further delineate the contribution of these cytokine pathways to DSS-induced colitis, we quantified the serum levels of 17 inflammatory cytokines, as an indicator of the systemic inflammatory pathways induced upon gut inflammation. Only six of the 17 cytokines were reliably detected in the serum of DSS-treated mice, namely, IFN-γ, TNF-α, IL-1β, IL-6, IL-18 and IL-22 (Fig. 2, Supplementary Fig. 2, and data not shown). The IL-12 and IL-23 pathways respectively induce the production of IFN-γ and IL-22 [31,32]. In agreement with this, we find a slight reduction in serum IFN-γ levels in IL12Rb2$^{-/-}$ relative to B6 mice (Fig. 2). In addition, the level of IL-22 was almost undetectable in IL23R$^{-/-}$ mice upon DSS-treatment (Fig. 2). Interestingly, DSS-treated IL10$^{-/-}$ mice exhibited a significant increase in circulating pro-inflammatory cytokines namely IFN-γ, TNF-α and IL-1β. Together, these results suggest that IL-10, IL-12 and IL-23 pathways each differentially contribute to DSS-induced inflammatory response.

3.3. IL-12 and IL-23 pathways play opposing roles in the context of IL-10-deficiency

To further characterize the role of these cytokines in IBD and to determine whether the combined deficiency of IL-10 and either IL-12 or IL-23 would synergize or antagonize the development of DSS-induced colitis, we generated IL10$^{-/-}$.IL12Rb2$^{-/-}$ and IL10$^{-/-}$.IL23R$^{-/-}$ double deficient mice. Interestingly, compared to IL10$^{-/-}$ mice, IL10$^{-/-}$.IL12Rb2$^{-/-}$ mice exhibited less weight loss, while IL10$^{-/-}$.IL23R$^{-/-}$ mice lost significantly more weight leading to a > 20% decrease in body weight after 7 days of DSS treatment (Fig. 3a and b). The changes in body weight again paralleled colitis severity as evaluated on histological colon sections showing that IL10$^{-/-}$.IL23R$^{-/-}$ mice displayed the most severe lesions with notable ulceration (Fig. 3c). As intestinal crypts structures were lost, it was not possible to provide quantify crypts dilation or goblet cell loss for these severely affected mice (Fig. 3c). Together, these results suggest that IL-12 and IL-23 pathways have distinct roles in the context of IL-10-deficiency.

We next evaluated the systemic inflammatory profile of these mice, which were induced following DSS-treatment in IL10$^{-/-}$, IL10$^{-/-}$.IL12Rb2$^{-/-}$, IL10$^{-/-}$.IL23R$^{-/-}$ mice (Supplementary Fig. 3). As shown in Fig. 4, and in agreement with a reduction in weight loss in IL10$^{-/-}$.IL12Rb2$^{-/-}$ mice relative to IL10$^{-/-}$ mice, IL10$^{-/-}$.IL12Rb2$^{-/-}$ mice exhibited lower levels of IFN-γ and IL-1β. This result suggests that the IL-12 pathway significantly contributes to systemic cytokine levels in DSS-induced colitis in IL10$^{-/-}$ mice. Conversely, we observed a reduction in IL-18, IL-22 and IL-1β in IL10$^{-/-}$.IL23R$^{-/-}$ mice relative to IL10$^{-/-}$ mice (Fig. 4). As IL-22 is important for maintaining epithelial integrity [33], it is tempting to suggest that the exacerbated weight loss in DSS-treated IL10$^{-/-}$.IL23R$^{-/-}$ mice relative to IL10$^{-/-}$ mice is a consequence of the absence of induction of IL-22. Taken together, these data highlight the opposing roles of IL-12 and IL-23 in regulating the systemic inflammatory cytokine response in DSS-induced colitis.

3.4. IL-12Rβ2 and IL-23R deficiencies protect IL10$^{-/-}$ mice from spontaneous colitis

DSS is a chemically induced colitis model that disrupts the epithelial barrier [34]. In contrast, IL10$^{-/-}$ mice will spontaneously develop colitis around 25 weeks of age as a result of an imbalance in the immunoregulatory immune response [16]. Although both models mimic significant aspects of human IBD pathology [34], the progression to disease is mediated by distinct pathways [35]. To address whether IL-12 and IL-23 pathways have conserved roles in regulating the inflammatory response in the absence of IL-10, we examined the occurrence of spontaneous colitis in cohorts of IL10$^{-/-}$, IL10$^{-/-}$.IL12Rb2$^{-/-}$ and IL10$^{-/-}$.IL23R$^{-/-}$ mice. The mice were aged at least for 25 weeks and the degree of colitis was quantified by histology. As expected, mice deficient in either IL-12Rβ2 or IL-23R alone did not show signs of colitis (data not shown), while 33% of IL10$^{-/-}$ mice showed significant histological inflammatory lesions in the colon (Fig. 5). When compared with IL10$^{-/-}$ mice, IL10$^{-/-}$.IL12Rb2$^{-/-}$ mice were

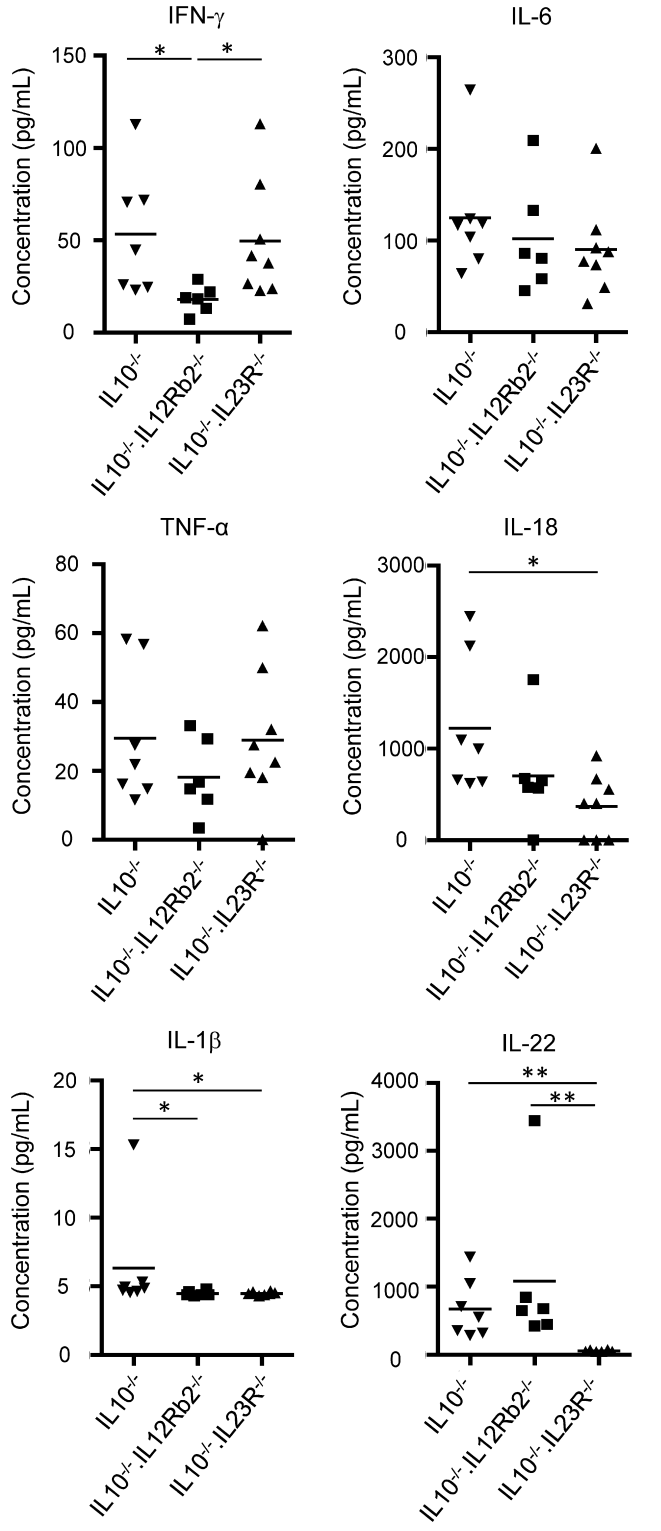

Fig. 4. Deficiency in either IL-23R or IL-12Rβ2 alters the cytokine profile of IL10$^{-/-}$ mice. Cytokine levels in the serum from IL10$^{-/-}$ (inverted triangle, n = 7), IL10$^{-/-}$.IL12Rb2$^{-/-}$ (square, n = 6) and IL10$^{-/-}$.IL23R$^{-/-}$ (triangle, n = 10) DSS-treated mice was quantified using the Cytokine 17-Plex Mouse ProcartaPlex™ Panel. For all panels, the horizontal bar represents the mean for each group and the dot represents one mouse sample. *P* values, * < 0.05; ** < 0.01. IL-1β levels remain statistically elevated in IL10$^{-/-}$ mice relative to the other strains, even upon removing the elevated data point for IL-1β in IL10$^{-/-}$ mice (data not shown). IL-22 levels also remain statistically increased in IL10$^{-/-}$.IL12Rb2$^{-/-}$ mice relative to IL10$^{-/-}$.IL23R$^{-/-}$ mice, even upon removing the elevated data point for IL-22 in IL10$^{-/-}$.IL12Rb2$^{-/-}$ mice (data not shown).

protected against the spontaneous occurrence of colitis. Surprisingly, and in contrast to DSS-induced colitis, IL10$^{-/-}$.IL23R$^{-/-}$ mice were also protected against the occurrence of spontaneous colitis (Fig. 5). Thus, deletion of either IL-12 or IL-23 mitigates the development of spontaneous colitis suggesting that both pathways contribute to IBD in IL10$^{-/-}$ mice.

To better understand how the IL-12 and IL-23 pathways modulated the systemic inflammatory response in the spontaneous colitis model of IL-10-deficiency, we again analyzed 17 cytokines in the mouse serum. In this spontaneous colitis model, we robustly detected nine cytokines in the serum of IL10$^{-/-}$ mice (Supplementary Fig. 4). Compared to B6 mice, IL10$^{-/-}$ mice showed increase levels of IFN-γ and IL-17 (Supplementary Fig. 4). In line with the protective role of IL-12Rβ2 deficiency, when compared with IL10$^{-/-}$ mice, mice lacking both IL-10 and IL-12Rβ2 showed a decrease in IFN-γ, IL-17a, TNF-α, and IL-13 (Fig. 6). Similarly, IL10$^{-/-}$.IL23R$^{-/-}$ mice exhibited reduced levels in IFN-γ, IL-17a, TNF-α and IL-22 relative to IL10$^{-/-}$ mice (Fig. 6). Taken together, these data show that IL-12Rβ2 and IL-23R signaling provokes distinct but overlapping alteration in cytokine levels. Altogether, inhibition of IL-12 and IL-23 signaling decrease the spontaneous occurrence of colitis, with each pathway modulating unique cytokine profiles. These results further highlight the distinct roles of IL-12 and IL-23 in the ongoing systemic inflammatory response occurring during the spontaneous development of colitis in IL10$^{-/-}$ mice.

4. Discussion

IBD is a complex inflammatory disease, where an imbalance in cytokine production contributes to disease pathogenesis. Of those, IL-12 and IL-23 pathways have been recognized as important risk factors for gut inflammation [3,36]. IL-10 also plays a critical role in gut homeostasis and loss of function of the IL-10 signaling pathway has been implicated in early onset IBD [14,15]. Considering the importance of these cytokines in IBD, our aim was to delineate the contribution of IL-12 and IL-23 and their interrelationship with IL-10 in colitis development using two different models: DSS-induced and IL10$^{-/-}$ mice-spontaneous colitis. In DSS-induced colitis, chemical damage of the epithelial barrier leads to increased intestinal permeability and immune cell infiltration. In spontaneous IL10$^{-/-}$ mouse colitis model, the intestinal flora initiates inflammation and alteration of the gut epithelium [16].

Our study demonstrates that deficiency in IL-12Rβ2 or IL-23R alone, or IL-10 and IL-12Rβ2 in combination, did not increase the severity of DSS-induced colitis, whereas the combined deficiency of IL-10 and IL-23R cooperates to exacerbate weight loss and gut ulceration. In contrast in the spontaneous IL10$^{-/-}$ mouse model, deficiency in either IL-12Rβ2 or IL-23R protected against colitis. Overall, these results highlight the complex interactions between IL-10 and IL-12 family cytokines in the development of colitis in IBD.

Our data show that IL10$^{-/-}$ mice are more susceptible to DSS treatment, leading to increased weight loss, systemic inflammatory cytokines levels (IFN-γ; TNF-α; IL-1β), and intestinal inflammation. IL-10 is an anti-inflammatory cytokine that plays an important role in the suppression of the effector function of Th1 and Th17 cells as well as NK cells and macrophages [37]. Administration of IL-10 prior to DSS treatment reduces colitis severity and improves the inflammatory profile [38,39]. Interestingly, the combined deficiency of IL-10 and IL-23R, but not of IL-10 and IL-12Rβ2, exacerbated DSS-induced colitis compared to IL10$^{-/-}$ mice. The distinct roles of the IL-12 and IL-23 pathways in DSS-induced colitis may be attributable to the fact that IL-12Rβ2 and IL-23R are expressed on different immune cell types leading to specific cytokine production [9]. For instance, deficiency in IL-23R signaling is associated with a complete lack of IL-22 production, a cytokine that can induce detrimental as wells as protective effects on the intestinal barrier depending, in part, on the environmental milieu [40]. As such, to explain the exacerbation of colitis in IL10$^{-/-}$.IL23R$^{-/-}$

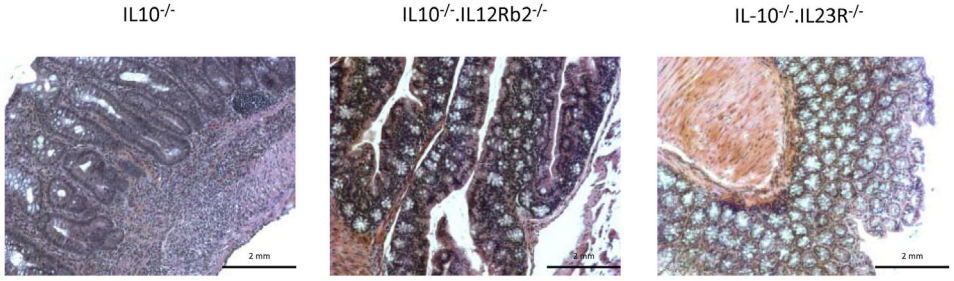

Fig. 5. Deficiency in either IL-12Rβ2 or IL-23R protects IL10$^{-/-}$ mice from the development of spontaneous colitis. Histological analysis of spontaneous colitis in IL10$^{-/-}$, IL10$^{-/-}$.IL12Rb2$^{-/-}$ and IL10$^{-/-}$.IL23R$^{-/-}$ mice by H&E-stained colon sections. Immune cell infiltration and goblet cell loss are observed in IL10$^{-/-}$ mice with evident ulceration (100×).

mice, it is tempting to suggest that IL-22 production in response to IL-23 alleviates the disruption of the epithelial barrier in DSS-treated IL10$^{-/-}$ mice. Regardless, our results support distinct roles for IL-12Rβ2 and IL-23R in DSS-induced colitis in IL10$^{-/-}$ mice.

In contrast to the DSS model, defects in either IL-12 or IL-23 signaling protects against spontaneous colitis development in IL10$^{-/-}$ mice. For both IL10$^{-/-}$.IL12Rb2$^{-/-}$ and IL10$^{-/-}$.IL23R$^{-/-}$ mice, this protection was associated with a reduction in circulating pro-inflammatory cytokine levels including IFN-γ, TNF-α and IL-17a, consistent with a decrease in the overall inflammatory signature. In addition to this common signature, both IL10$^{-/-}$.IL12Rb2$^{-/-}$ and IL10$^{-/-}$.IL23R$^{-/-}$ mice show some unique alterations in cytokine levels. Surprisingly, IL10$^{-/-}$.IL12Rb2$^{-/-}$ mice exhibited less IL-13, a Th2 cytokine, suggesting that the IL-12 cytokine promotes Th2 inflammation in IL10$^{-/-}$ mice, reminiscent of the type 2 inflammatory signature that has been reported in colitis [41]. Additional studies will be needed to determine how the presence of IL-12 facilitates the production of IL-13 in this context. Whereas IL-13 levels are not decreased in IL10$^{-/-}$.IL23R$^{-/-}$ mice, we expectedly noted a much reduced level of IL-22. In contrast to DSS-induced colitis, the low abundance of IL-22 is linked with a decrease in disease severity in the spontaneous colitis model. The opposite roles of IL-23 observed in IL10$^{-/-}$.IL23R$^{-/-}$ mice in the induced and spontaneous colitis models may be explained by the mechanism underlying colitis development. In the DSS model, disruption of the epithelial barrier is the main factor implicated in intestinal inflammation and colitis, where IL-22 production following IL-23 response could have a protective role [33,40,42]. In the spontaneous model, the immune system is required to initiate the disease, and IL-23 signaling, which contributes to differentiation of pathogenic Th17 cells, could promote colitis [27]. These IL-23R specific effects have been reported previously. Indeed, lack of IL-23R on innate cells protected against colitis in a Rag$^{-/-}$ model, whereas it exacerbated the disease in a T cell driven model [42]. Moreover, treatment with anti-IL-23R blocking antibody was protective in a T-cell transfer colitis model [27], while specific IL-23R-deficiency in colonic epithelial cells exacerbated the DSS-induced inflammation [43,44]. The distinct response to IL-23R deficiency in different models of colitis highlights the complex interaction among cytokines but also with environmental factors leading to colitis. Our study shed light on potential mechanism implicated in the etiology of IBD.

Of note, in this study, we quantify the serum cytokines as opposed to the local intestinal cytokine production. We correlate these finding with weight loss and histological scores to determine the severity of the intestinal inflammation. Although this is a limit of our study, others have shown that the profile of pro-inflammatory cytokines can be used as a proxy for disease activity both in patients and mouse models of IBD [45–47]. Furthermore, methods using panels of circulating pro-inflammatory cytokines has allowed for the identification of subgroups of IBD patients [48] and the cytokine profiles correlate with disease activity/severity [49]. Together, these results suggest that circulating cytokines can be used to monitor disease activity.

In conclusion, our data showed that in the context of IL-10 deficiency, IL-12Rβ2 is protective while the effect of IL-23 depends on the colitis model, triggered either by a breach in epithelial barrier (DSS induced-colitis) or as a consequence of an uncontrolled inflammatory response (IL10$^{-/-}$ mice spontaneous-colitis). Altogether, our work highlighted the complex interaction among various pro and anti-inflammatory cytokines in the development of IBD. The fact that the impact of IL-23 pathway on colitis severity is context-dependent may help explain the negative outcomes of anti-p40 treatment in some patients. Indeed, in the context where inflammation has resulted in severe epithelial destruction, it may be beneficial to provide IL-22 in addition to anti-p40 to circumvent the negative effects associated with targeting the IL-23 pathway. Additional studies are needed to better understand the patient context where anti-p40 would be beneficial, or whether specifically targeting IL-12 or IL-23 would lead to fewer adverse events.

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Declaration of Competing Interest

The authors declare that they have no competing financial interest in relation to the work described.

Author contribution

Raphaël Hurtubise: the conception and design of the study and data acquisition.

Cindy Audiger: statistical analysis; data analysis and interpretation and writing of the manuscript.

Maria C. Dominguez-Punaro; data analysis and interpretation; revised the manuscript.

Geneviève Chabot-Roy: data acquisition.

Gaëlle Chognard: the conception and design of the study and data acquisition.

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Lise Coderre: statistical analysis; writing of the manuscript.

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Stephen W. Michnick: the conception and design of the study, revised the manuscript.

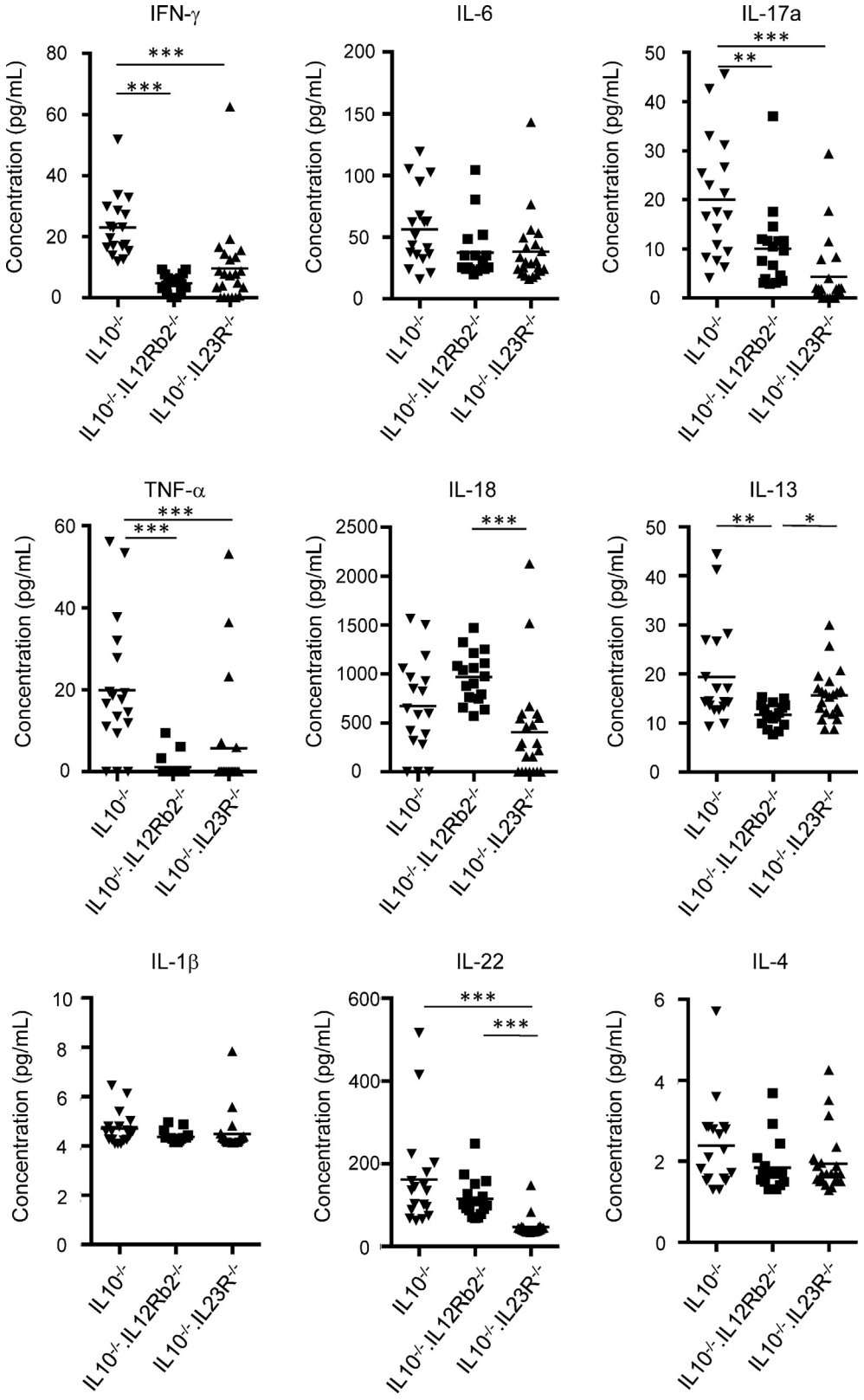

Fig. 6. Deficiency in either IL-12Rβ2 or IL-23R is associated with a decrease in the pro-inflammatory cytokine profile in IL10$^{-/-}$ spontaneous colitis mouse model. Serum of IL10$^{-/-}$ (inverted triangle, n = 18 mice), IL10$^{-/-}$.IL12Rb2$^{-/-}$ (square, n = 17 mice) and IL10$^{-/-}$.IL23R$^{-/-}$ (triangle, n = 22 mice) was collected from 28 week-old mice. Cytokines were quantified with the Cytokine 17-Plex Mouse ProcartaPlex™ Panel. For all panels, the horizontal bar represents the mean for each group and the symbol represents one mouse sample. *P* values, $^{*} < 0.05$; $^{**} < 0.01$; $^{***} < 0.001$.

John D. Rioux: the conception and design of the study, revised the manuscript.

Sylvie Lesage: the conception and design of the study, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, final approval of the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.154738.

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